EVALUATION AND COMPARISON OF THE ANTIOXIDANT AND FREE RADICAL SCAVENGING PROPERTIES OF MEDICINAL PLANTS BY USING THE DPPH ASSAY *IN-VITRO*

Short running head: Antioxidant and radical scavenging properties of medicinal plants

Impacts/ Highlights

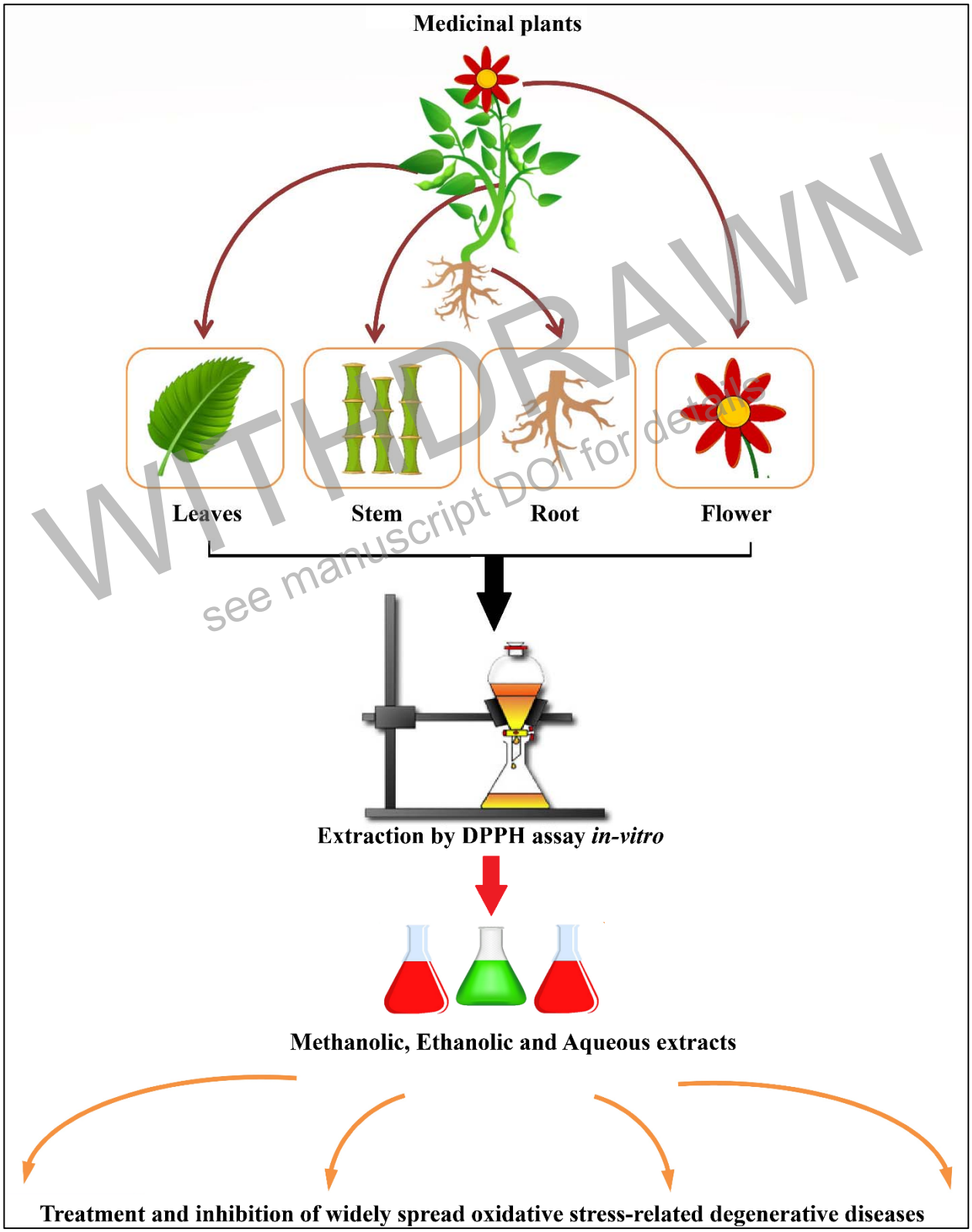
- Samples in ethanolic extracts showed a higher value of radical scavenging potential.
- The highest radical scavenging activity was observed in the *Euphrasiae stricta* (IC_{50}= 38.972 µg/mL), *Euphorbia platyphyllos* L. (IC_{50}= 40.817 µg/mL) and *Epimedium brevicomum* Maxim (IC_{50}= 46.265 µg/mL).

Abstract

Introduction: Free radicals have been found to cause a number of problems and damage in the human body especially cardiovascular disorders and cancer. These radiation-based treatments may have some damaging effects on other normal body cells. So there is a need to explore some natural means which may contain antioxidants, which can trap the ROS produced within the living body. *Methods:* In this research, various medicinal plant samples were observed for free radical hunting or scavenging activity of their methanolic, ethanolic and aqueous extracts by using the DPPH (1, 1- diphenyl, 2-picryl-hydrazyl) assay in-vitro by taking the absorbance reading at 517nm using a spectrophotometer, because of flavonoids and other polyphenol contents, the anti-oxidant activity was possessed by these traditionally used medicinal plants from Himalayan regions of Pakistan. *Results:* The results showed that all samples in ethanolic extracts showed a higher value of radical scavenging potential. The highest radical scavenging activity was observed in the *Euphrasiae stricta* (IC_{50}= 38.972 µg/mL), *Euphorbia platyphyllos* L. (IC_{50}= 40.817 µg/mL), and *Epimedium brevicomum* Maxim (IC_{50}= 46.265 µg/mL), medicinal plants for both of their ethanolic and methanolic extracts as compared to the Ascorbic Acid scavenging activity (IC_{50}=37.337 µg/ml). *Conclusion:* These Plants can be efficiently applied as an important antioxidant source for the treatment and inhibition of widely spread oxidative stress-related degenerative diseases like cancer, cardiovascular & inflammatory disorders, atherosclerosis, dementia, diabetes, asthma, and eyes related to degenerative diseases, etc.

Keywords: free radical, scavenging, antioxidant, medicinal plants, Pakistan

Graphical abstract



INTRODUCTION

It has been investigated that the reactive oxygen species (ROS) in human body, e.g., H_2O_2, O^{2-} and OH^- are found in large amount. These may be primarily because of the reason that almost 5% or more oxygen (O_2) which is inhaled by a human is transformed to these ROS because of univalence reduction of O_2.[1, 2] Various important diseases such as diabetes, cancer, cirrhosis, obesity and cardiovascular disorders may be caused by these free radicals. [3] So several enzymatic antioxidant barriers like super oxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) have been efficiently used to neutralize these harmful properties of these free radicals. However, to investigate this oxidative stress, certain factors like ultraviolet rays, unnecessary NADPH stimulation, and cigarette smoke, environmental contaminants/pollutants exposure, mitochondrial electron transport chain, radiation, some parasitic infections or toxic chemicals have been focused and are responsible in causing the overproduction of the ROS. The oxidative stress is a change in the equilibrium/normal position of an antioxidant or pro-oxidant processes in a living systems, resulting in the mutilation/harm to different component like DNA, lipids and membranes proteins and collectively to whole cell structures.[4, 5] So, these diseased condition can be effectively improved by compounds called antioxidants that can hunt and neutralize the free radicals.[6] An extensive diversity of free radical hunting or antioxidant components like phenols, vitamins, terpenoids and flavonoids have been found in plants which possess high antioxidant potentials.[7] The plant derived polyphenolic constituents might be more useful *in-vivo* with their positive effects as these are proved to be more efficient antioxidants as compared to vitamins E or C *in-vitro*.[8]Different medicinal plants had been efficiently applied to treat the ROS and are of great importance having antioxidant potential because of such sort of alimentary radical scavenging diet supplement. The antioxidant potentials of medicinal plants are mainly because of rich source of phyto-nutrients and ingredients like phenols, flavonoids and terpenoids present in them. The antioxidant potentials of many medicinal plants have been studied [9, 10] like anti-cancer and Immunomodulator activity, [11] Hepato-protective benefits, hypolipidemic activity [12]. Hence, the free radical scavenging activity by evaluation and comparison of various medicinal plants with different protocols using spectrophotometer was the main aim of the present study. [13, 14]

MATERIALS AND METHODS

Materials

The Medicinal plant samples were collected from Himalayan regions of Pakistan and were identified by Professor Dr. Mohammad Ibrar Shinwari, Chairman Department of Environmental Sciences, International Islamic University, H-10, Islamabad (IIUI), and Pakistan. The dried samples were carried out to University of Virginia (UVa), Department of Biology, USA where the evaluation antioxidant properties of the medicinal plant samples was achieved under the supervision of Professor Dr. Michael P. Timko. The chemicals, solvents and reagents

used in the preparation of plant extracts were Distilled water, Ascorbic acid, DPPH (1, 1-diphenyl, 2-picryl hydrazyl), Methanol and Ethanol.

Table 1: Traditionally used Medicinal Plants collected from Himalayan regions of Pakistan

Sr. No.	Scientific Name	Plant Pictures	Local Name	Family	Parts Used	Traditional Medicinal Uses
1.	*Trifolium repense* L.		White Clover, Desi siree, Saag	Fabaceae	Leaves	Stomach problems, Anti-intestinal helminthic worms, Anti-cestodal properties
2.	*Cassia tora* Linn		Kikkar, Cassias	Fabaceae	Leaves, Seeds	Phyto-chemical and Pharmacological Properties
3.	*Psoralea corylifolia* L.		Babchi or Bakochi	Fabaceae	Leaves, Seeds	Antibacterial, antitumor, anti-inflammatory and immunomodulatory activity.
4.	*Urticae folium*		Nettles or Stinging nettles,	Urticaceae	Seeds, Leaves	Antimicrobial, antiulcer and analgesic activities
5.	*Teraxaci folium*		Dandelion	Asteraceae	Whole Plant Extract	Anti-inflammatory, anti-carcinogenic and antioxidative activities

6.	*Rubi idaei folium*		Akhriyar, Jammaro	Phragmidiaceae	Fruits, leaves	Antimicrobial properties
7.	*Rhus potaninii* Maxim		Desi drawa,Tunn	Anacardiaceae	Fruits, leaves	Anti- hypertension to control high BP, Anti-diabetic properties
8.	*Lantana camara*		Big-sage, wild-sage, red- sage, white-sage, tick-berry	Verbenaceae	Whole Plant Extracts	Antimicrobial, fungicidal, insecticidal, Anti- cancer, skin itches, leprosy, rabies, chicken- pox, measles, asthma and anti-ulcer properties.
9.	*Lonicera japonica Thunb.*		Chanbha. Japanese honeysuckle and golden and silver honeysuckle	Caprifoliaceae	Dried leaves, Stem and flowers	Anti-inflammatory, to treat fever, headache, cough, thirst, and sore throat
10.	*Epilobii herba*		Willowherbs	Onagraceae	Whole Plant Extracts	Used for prostate hyperplasia (BPH),bladder and hormone disorders

11.	*Thymus serpyllum* L.		Breckland thyme, Breckland wild and creeping thyme	Lamiaceae	Aerial Parts	Antimicrobial, treating fever and antitumor activity
12.	*Salvia officinalis*		Garden sage, common sage, or culinary sage	Lamiaceae	Whole Plant Extract	Treating Alzheimer's Disease as neurotoxic
13.	*Euphrasiae herba*		Eyebright	Orobanchaceae	Whole Plant Extract	For eyestrain and to relieve inflammation caused by colds, coughs, sinus infections, sore throats and hay fever.
14.	*Equiseti herba*		Horsetail, snake grass, puzzle grass	Equisetaceae	Aerial parts	Antimicrobial and Geno-toxicity
15.	*Millefolii herba*		Yarrow or common yarrow	Asteraceae	Leaves, Flowers	Antimicrobial Activity
16.	*Mentha piperita* folium		Wild Mint	Lamiaceae	Whole Plant Extract	Antimicrobial Activity

17.	*Euphorbia platyphyllos* L.		Dhoodal	Euphorbiaceae	Aerial parts	Cytotoxic and apoptotic activities
18.	*Marrubium vulgare*		White horehound	Lamiaceae	Aerial parts	Treating stomach problems
19.	*Melissae folium*		Lemon Balm, balm, common balm, or balm mint	Lamiaceae	Whole Plant Extract	Antimicrobial Activities
20	*Hederae folium*		Jal bail	Araliaceae	Leaves, Fruit	Antimicro, Antioxidative, hepato-protective and Antimutagenic activities
21	*Fritillaria thunbergii*		Lilly	Liliaceae	Whole Plant Extract	Antimicrobial activities
22	*Satureja montana*		Mountain savory, winter savory	Lamiaceae	Whole Plant Extract	Antimicrobial activities

23	*Gunnera perpensa*		River pumpkin, wild rhubarb, wild ramenas, Nalcas.	Gunneraceae	Stem, Leaves	Anti-microbial, anti-inflammatory and anti-oxidative properties
24	*Absinthii herba*		Absinthe, Absinthim, absinthe wormwood, grand wormwood, wormwood	Asteraceae	Stalk, Leaves	Antidepressant and Antioxidant activities
25	*Viscum album*		Mistletoe	Santalaceae	Leaves, fruit, Stalk	Antihyperglycemic activity
26	*Asperula Herba*		Woodruff, Golden rod	Rubiaceae	Leaves, stalk, Flowers	Antibacterial activities
27	*Tephrosia purpurea* L.		Sarphonk, Sharpunkha, Fish poison, Wild indigo	Fabaceae	Leaves	Anticarcinogenic and Anti-lipid peroxidative effects
28	*Tinospora cordifolia* (Willd.)		Heart-leaved moonseed, guduchi and giloy	Menispermaceae	Leaves	Anti- bacterial, Antifungal Properties

29	*Ocimum basilicum*		Basil, sweet basil	Lamiaceae	Leaves	Antioxidant and anticancer Properties
30	*Saussurea lappa*		Costus or kuth	Asteraceae	Whole Plant	For stomach problems
31	*Betulae folium*		Silver birch, warty birch, European white birch, or East Asian white birch	Betulaceae	Bark, Leaves	Anti-inflammatory, antiviral and anti-cancer properties.
32	*Cantaurii herba*		Centaury, Centory, Starthistles, knapweeds, Centaureas	Asteraceae	Whole Plant	Cytotoxic, antifungal and antimicrobial properties
33	*Prunella vulgaris*		Common self-heal, woundwort, carpenter's herb, brown-wort and blue curls	Lamiaceae	Leaves, Flower	Cytotoxic and immunomodulatory activities
34	*Echter ehrenpreis*		Speedwell, Common Gypsy-weed	Veroniceae	Whole Plant	Antimicrobial Properties

35	*Paris polyphilla*		Love Apple, Satuwa	Melanthiaceae	Leaves	Antimicrobial Properties
36	*Melissa officinalis* L.		Lemon balm, balm, common balm, or balm mint	Lamiaceae	Leaves, Stalk	Genotoxicity and cytotoxicity
37	*Hedyotis diffusa*		White flower, snake-tongue grass.	Rubiaceae	Whole Plant Extract	Anti-inflammatory, cytotoxic and Antibacterial activities
38	*Polygonum aviculare*		Common knotgrass, Prostrate knotweed, Bird-weed pigweed and low-grass	Polygonaceae	Leaves, Stalk	Antimicrobial and Anti-inflammatory Properties
39	*Salviae off*. folium		Sage, also called garden sage, common sage, culinary sage.	Lamiaceae	Aerial parts	Cytotoxic Properties

40	*Fagopyrum cymosum*		Buckwheat, Tartary buckwheat	Polygonaceae	Aerial Parts	Anti-inflammatory also to treat fever, headache

Methodology: Preparation of Medicinal Plant Extracts

For preparing the methanolic extracts, 100 mg of dried powdered plant leaf samples were taken in 1.5ml of aqueous Methanol (40%) in Eppendorf tubes. The samples were shaken well to dissolve to greater extent with the help of stirrer/shaker. All samples were then incubated in dark for 24 hours. The extracts were then filtered to remove undissolved solid particles in form of pellet and the supernatant was obtained for further analysis.

Preparation of 1mM DPPH solution

For preparing 1mM of DPPH solution, 3.94mg of DPPH was added in 100 ml methanol in a 200 mL flask and was well mixed with the help of stirrer. DPPH gave purple color when dissolved in Methanol. The incubation was then done at room temperature for 20 min. The series of dilution were made for less concentrated DPPH solution, as highly concentrated solution does not give accurate absorbance value by blocking most of light in spectrophotometer. Different concentrations of plant extracts (5 µg, 25ug, 50µg, 75µg and 100 µg) were added in diluted DPPH solution. The samples were kept in incubation for 30 min in dark again. The purple color of DPPH in Methanol solution was faded or completely disappeared because of the activity of antioxidants from medicinal plant samples. The free radical hunting or scavenging activity was measured by taking the absorbance reading of samples at 517 nm on spectrophotometer.

The methanol, as the basic and the DPPH were used as a blank and negative control respectively. Ascorbic Acid was taken as standard because of its high Antioxidant activity. The increased absorbance indicated the high antioxidant activity of samples. The formula used to calculate the reduction %age was,

%age reduction= Ac-As/Ac*100

Preparation of Ascorbic Acid Stock solution

To prepare the standard Ascorbic Acid stock standard solution, 0.5mg of Ascorbic Acid was mixed in 1 mL of Methanol.

Ascorbic Acid Curve graph

The %age reduction in absorbance was observed using different concentration of Ascorbic Acid in sample solution to construct an Ascorbic Acid curve. The Eppendorf tubes were taken with 40uL of DPPH in 1.5 mL of Methanol by mixing it well on stirrer. The initial absorbance reading was taken at 517nm.

The preparation of Ascorbic Acid solution (standard) was done by dissolving the0.5mg/mL of Ascorbic acid in 1mL of methanol. To measure the absorbance difference, different ranges of this solution i.e., (10-100 uL), were added to DPPH sample solution. The DPPH reagent solution was taken as a control in separate Eppendorf tubes. The absorbance value of the samples was then measured at 517nm, after 5 minutes of incubation at room temperature using spectrophotometer.

The %age reduction/inhibition was calculated by using following formulae,

%age Inhibition= Ac-As/Ac*100

In this formula Ac=Absorbance of control and As=Absorbance of Sample

A graph was plotted between "%age Absorbance inhibition vs Concentration". The IC_{50} for Ascorbic Acid and plant samples was also calculated.

IC50 value

The Inhibition concentration or IC_{50} is the value which shows the half or 50% inhibition or reduction in initial absorbance by an antioxidant in DPPH Assay. It was calculated by plotting different concentration of extracts and by reading their % inhibition/reduction. The lower IC_{50} indicated the antioxidant potential of the sample.

Calculation of IC_{50} value in Antioxidant assays.

It was a simple calculation consisting of following steps;

A scattered graph was made in excel (where X axis was taken as concentration and Y axis as %age Inhibition activity)

The slope equation used was (Y=mx+c or Y=mx-c)

Where the value of "Y" in IC_{50}equation was Y=50or (0.5) as it means the half or 50% inhibition/reduction in absorbance of samples.

In next step, the IC_{50} of methanolic, ethanolic and aqueous extracts of all medicinal plant samples was also measured following same DPPH protocol for methanol (2 mg/ml), 0.5 mg/mL of ethanol and water (2 mg/mL) with the addition of increasing concentration i.e., methanol (5-100 µl), Ethanol (10-50 µl) and water (5-100 µl) in methanol and DPPH reagent solution in already prepared Eppendorf tubes.

Table 2: Reduction in the percentage of DPPH absorbance values of samples at 517 nm with the addition of Ascorbic Acid.

Sr. No.	Concentration (ug/mL)	Absorbance	%age Inhibition	IC_{50} (ug/mL)	R^2
1	10	0.944	34.4459		
2	20	0.884	38.623924		
3	30	0.752	47.7789		
4	40	0.691	52.1149		
5	50	0.616	57.22334	37.337	0.989
6	60	0.575	60.0695		
7	70	0.426	70.417		
8	80	0.394	72.69987		

9	90	0.242	83.195		
10	100	0.198	86.25		
Blank (Methanol)		**1.44**			

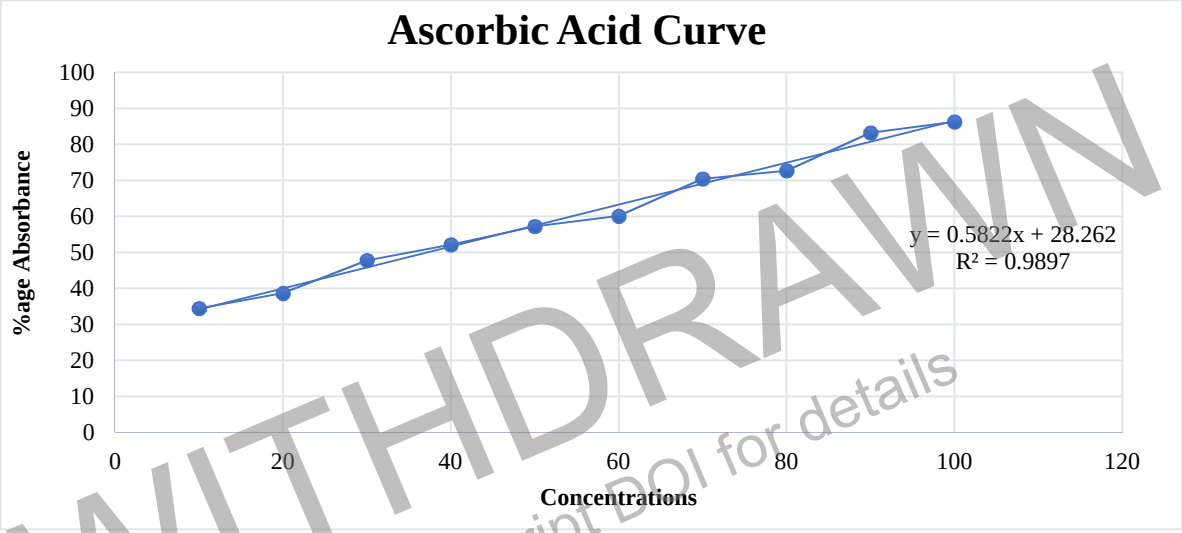


Figure 1: Graphical presentation of Ascorbic Acid Absorbance at 517nm.

RESULTS AND DISCUSSION

The DPPH solution was used in this experiment because it is never affected by some side reactions, like enzymatic suppression or metal ion chelation, comparing with the other free radicals like superoxide oxides and hydroxyl ions. The deep purple color was shown by freshly prepared DPPH solution with maximum absorbance at 517nm. The purple color of DPPH was faded up or almost disappeared because of the antioxidants activity present in these plant extracts. Therefore, the free radicals in DPPH can be neutralized by antioxidant molecules (i.e., by providing hydrogen atoms or donating electrons, possibly through an attack on the free radicals present in DPPH molecule) and thus resulting in purple to colorless change in color (e.g., by converting to 2, 2-diphenyl-1-hydrazine, or by replacing corresponding hydrazine molecule), which showed increase in the absorbance at 517nm. This DPPH Assay is also very useful as the increase in absorbance of the sample solution can be directly measured by a continuous spectrophotometry in the reaction medium at any time. The consistent information regarding the antioxidant potential of these tested plant samples has been efficiently measured using DPPH assay [15, 16].

In this research, the ethanolic, methanol and aqueous plant extracts of 40 medicinal plants were experimented to test their free radical scavenging potential using DPPH assay. The “Table 2” and “Figure 2” showed the IC_{50} (the test solution concentration required to increase the absorbance of a sample by 50% comparing to the blank solution) for different medicinal plants in different extracts. The results showed that ethanolic extracts of these medicinal plant extracts

showed the higher level of free radical scavenging or antioxidant properties, followed by methanolic extracts in comparison with the IC_{50}=37.337µg/ml of standard ascorbic acid. The ethanolic and methanolic extracts *Euphrasiae stricta* L. showed the highest antioxidant potential of 38.972ug/mL and 43.665ug/mL respectively followed by the *Euphorbia platyphyllos* L. (40.817 ug/mL & 42.988 ug/mL) and *Epimedium brevicomum* Maxim (46.265 ug/mL & 51.249 ug/mL) as compare to the Ascorbic acid IC=37.337 ug/mL. The aqueous extracts showed almost similar results for all samples regarding %age inhibition of free radicals.

Thus, these medicinal plants with the higher antioxidant potentials can be efficiently used as an efficient source of natural antioxidants to treat various oxidative stress related problems like cancer and other cardiovascular disorders. A quality control protocol may also be designed by following this study obtained by DPPH assay method and might be useful to develop an efficient protocol to investigate the antioxidant, anticancer or phytochemical activities of traditionally used medicinal plants.

Table 3:Free Radical Scavenging Activity of Medicinal Plant samples using different Extract Solutions.

Sr. No.	**Plant Sample**	**Sample Extracts**	**Radical Scavenging Activity (RSA) IC_{50}** (ug/mL)	**R^2**
1	*Euphrasiae stricta*	Ethanol	38.972	0.968
		Methanol	43.665	0.962
		Water	110.057	0.985
2	*Euphorbia platyphyllos* L.	Ethanol	40.817	0.983
		Methanol	42.988	0.979
		Water	121.512	0.997
3	*Epimedium brevicomum* Maxim.	Ethanol	46.265	0.978
		Methanol	51.249	0.996
		Water	98.605	0.981
4	*Viscum album*	Ethanol	52.279	0.984
		Methanol	54.463	0.991
		Water	141.227	0.979
5	*Psoralea corylifolia* L.	Ethanol	51.821	0.988
		Methanol	52.665	0.995
		Water	124.134	0.999
6	*Equiseti arvense*	Ethanol	55.246	0.958
		Methanol	58.781	0.993
		Water	128.427	0.983
7	*Veronica officinalis*	Ethanol	51.594	0.998
		Methanol	54.159	0.983
		Water	113.361	0.984

8	*Artemisia herba*	Ethanol Methanol Water	53.036 55.514 107.481	0.954 0.996 0.976
9	*Fagopyrum cymosum*	Ethanol Methanol Water	54.801 57.882 125.838	0.981 0.991 0.981
10	*Prunella vulgaris*	Ethanol Methanol Water	53.667 54.531 104.822	0.985 0.996 0.964
11	*Hederae folium*	Ethanol Methanol Water	56.797 59.797 109.527	0.982 0.998 0.981
12	*Salvia Divinorum*	Ethanol Methanol Water	60.961 65.356 111.201	0.979 0.999 0.977
13	*Thymus serpyllum* L.	Ethanol Methanol Water	52.559 57.329 134.297	0.996 0.994 0.972
14	*Melissae officinalis*	Ethanol Methanol Water	51.768 59.436 59.436	0.952 0.997 0.995
15	*Cassia tora* L.	Ethanol Methanol Water	54.768 58.971 149.891	0.991 0.998 0.982
16	*Saussurea lappa*	Ethanol Methanol Water	55.289 58.978 112.452	0.988 0.999 0.995
17	*Epilobium parvifolium*	Ethanol Methanol Water	57.381 59.831 116.097	0.982 0.999 0.994
18	*Satureja montana*	Ethanol Methanol Water	57.335 61.972 113.874	0.991 0.996 0.987
19	*Asperula odorata*	Ethanol Methanol Water	64.561 66.287 117.113	0.973 0.974 0.988
20	*Gunnera perpensa*	Ethanol Methanol Water	57.705 59.522 144.375	0.987 0.998 0.974
	Fritillaria thunbergii	Ethanol	55.551	0.997

21		Methanol	58.624	0.998
		Water	123.577	0.977
22	*Melissa flava*	Ethanol	52.658	0.987
		Methanol	54.856	0.999
		Water	114.268	0.982
23	*Ocimum basilicum*	Ethanol	58.759	0.984
		Methanol	60.446	0.999
		Water	91.913	0.986
24	*Achillea millefolium*	Ethanol	57.279	0.979
		Methanol	58.336	0.981
		Water	118.324	0.998
25	*Urticae folium*	Ethanol	51.986	0.996
		Methanol	53.122	0.997
		Water	126.186	0.991
26	*Polygonum aviculare*	Ethanol	57.452	0.994
		Methanol	62.838	0.998
		Water	132.052	0.979
27	*Lonicera japonica Thunb.*	Ethanol	66.194	0.992
		Methanol	82.037	0.991
		Water	123.601	0.969
28	*Tinospora cordifolia* (Willd.)	Ethanol	50.966	0.989
		Methanol	53.372	0.998
		Water	127.955	0.991
29	*Paris polyphilla*	Ethanol	55.081	0.979
		Methanol	56.098	0.997
		Water	137.321	0.999
30	*Mentha piperita* folium	Ethanol	51.739	0.995
		Methanol	52.919	0.999
		Water	120.492	0.986
31	*Tephrosia purpurea* L.	Ethanol	50.955	0.981
		Methanol	54.951	0.999
		Water	117.435	0.981
32	*Marrubium vulgare*	Ethanol	55.541	0.998
		Methanol	65.398	0.997
		Water	113.849	0.983
33	*Lantana camara*	Ethanol	51.546	0.992
		Methanol	53.722	0.992
		Water	131.394	0.983
34	*Betulae folium*	Ethanol	52.297	0.991
		Methanol	56.297	0.991

		Water	129.645	0.999
35	*Teraxaci folium*	Ethanol Methanol Water	57.496 61.474 117.948	0.989 0.998 0.985
36	*Rubi idaei folium*	Ethanol Methanol Water	58.889 63.138 110.017	0.988 0.999 0.995
37	*Hedyotis diffusa*	Ethanol Methanol Water	54.642 58.837 121.351	0.987 0.999 0.989
38	*Smilax glabra* Roxb	Ethanol Methanol Water	56.638 60.301 109.511	0.994 0.998 0.999
39	*Trifolium repense* L.	Ethanol Methanol Water	52.734 56.442 123.492	0.998 0.999 0.973
40	*Cantaurii herba*	Ethanol Methanol Water	52.487 58.836 134.361	0.992 0.998 0.981
	Standard A. Acid	Methanol	37.337	0.989

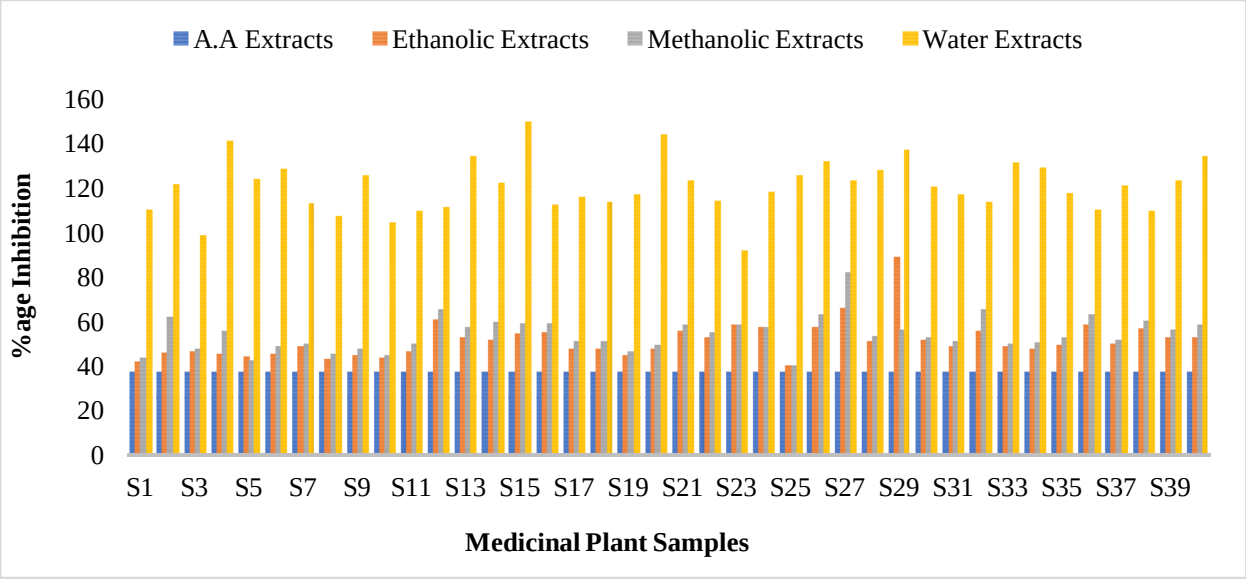


Figure-2: Graphical presentation of Free Radical Scavenging potential of various plant extracts of various Medicinal plant samples in comparison with Ascorbic Acid.

The *Euphrasiae stricta* plant was investigated for its antioxidant potentials in this research. It is an important medicinal plant in South Asia. Antioxidant assay was performed in

various solvent systems, i.e., water, ethyl ether, 70% ethanol and 80% methanol. The highest total phenol content were shown by the methanol extracts among all the extracts; so, these were used for additional research. To evaluate the antioxidant potential of these methanolic extracts various antioxidant assays were used like DPPH, metal ion chelating assay and FRAP Assay. Antibacterial activity and superoxide radical anions were taken as parameters. The antioxidant potential in the methanol extracts of *E. Stricta* sheets was comparable with strong antioxidants previously exploited and largely dependent on concentration [17].

The *Luphorbia platyphylos* L. (Euphorbiaceae) was investigated to verify the potential radical scavenging properties in its methanolic, petroleum ether, water, ethyl acetate and diethyl ether extracts using DPPH, DNA and cytotoxic activity in extracts of MCF-7 breast cancer cells from test excretion test exclusions assay DPPH trypan blue exclusion, Hoechst 33258 and comet assay double staining with iodide propidium, respective. From this study it was observed that all extracted samples showed a dose-response relation. Moreover, this study also showed that antioxidant properties were possessed by various extracts of *E. platyphylos* which caused DNA fragmentation. These results also proved that the breast cancer can be treated by using this plant as a potential source of anticancer agent. Further studies are needed to isolate and identify individual phenolic compounds in extracts [18].

The *E. pinnatum* extracts were evaluated to measure the total phenol content (TPC) and total flavonoids contents (TFC) through spectrophotometry methods. The higher antioxidant and antimicrobial activity was observed by the methanolic extract as compared to the ethanolic or water extracts. The methanol extract also showed high value of (149 and 36.6 mg /g) for TPC and TFC of *E. pinnatum* extracts. The ethanolic extracts showed antioxidant potential of (137.2) and 19.5 mg/g) followed by (86.2 and 8.4 mg /g) of water extracts. The lower IC value of 200 µg /mL was shown by methanolic extracts and then the (250 µg /mL) and (400 µg /mL) of ethanol and water extracts respectively. It was observed that there was a positive correlation found amongst antioxidant, antimicrobial activity, TPC and TFC in *E. pinnatum* extracts [19].

The potential antioxidant properties of methanol extracts of *Viscum album* ssp. (mistletoe) were investigated by using the 1.1-diphenyl-2-picrylhydrazyl (DPPH) assay to check the radical scavenging potential, FRAP and thiobarbituric acid were used to testify the lipid peroxidation inhibitory effect. The maximum activity was shown by the mistletoe extract grown in the lime tree in summer. It was also observed that harvest time and host tree had great influence on the plant's antioxidant capacity [20].

The ethanol extracts of *Psoralea corylifolia* seeds was studied for detection of their phytochemical properties. The total flavonoids and polyphenols were studied to check the presence of polyphenols. To determine the antioxidant activity1.1-diphenyl-2-picrylhydrazyl (DPPH) assay and superoxide radical assay were used. The concentrations of flavonoids and ethylene derivatives of polyphenol *Psoralea semicorylifolia* 60.63 QE mg/g and 74.35 mg/g respectively. The stronger antioxidant activity was shown by the extracts with a lower value of

IC_{50} for DPPH and the elimination of superoxide. The value of IC_{50} for DPPH and removal of phosphorus oxide was 166.61 mg/mL and 177.69 mg/mL, respectively. The strongest antioxidant activity of ethanolic extract was observed to be due to presence of flavonoids and phenols [21].

The antioxidant effects of different extracts of the *Equisetum arvense* L. (horse tail) were studied by using various antioxidant assays during lipid peroxidation of lipid particles. Antidepressant activity was studied in the human tumor cells of HLA, HT-29 and MCF7 using the sulforodamine B assay. It was confirmed from the analysis of these results that the ESR extracts suppress the formation of both lipid peroxidic roots and the investigation of adjuvant dosing systems. The results indicated that the extracts of ethyl ether, methanol, butanol and water extracts have been very effective in removing radical peroxigens. These findings showed that the *Equisetum arvense* extracts are a significant basis of voluntarily accessible accepted antioxidants and latent phytochemicals source [22].

The *V. teucrium* L., *V. officinalis* L. and *V. Orchidia* L. family *Plantaginaceae* were the three species tested for their potential phenolic, sterolic, antioxidant and antimicrobial activities. The quantification and identification of phenol compounds and other phytosterol were calculated using the p-coumaric and LC/MS techniques, folic acid, luteolin, and cytosterol acid the main components. More than this, Hespedolina, Yupatorean and Epatoline were first discovered in the genus Veronica. However, the content of the phytosterol of most of the Veronica genus were not examined. Antioxidant potentials that were examined through Trolox (TEAC) and EPR antioxidant assays showed that the *V. officinalis* and *V. Orchidea* showed comparable antioxidant potentials, while *V. Teucrium* extracts were recorded for lower values. These findings can be helpful promote the best use of genus Veronica as antioxidants and antimicrobial source [23].

The *Artemisia alba* herba (AHA) was tested for its potential antioxidant activity of its extracts by using the self-protective effect of powder against oxidation induced by alkanes in diabetic mice. The four groups were designed for mice random division: the first group receiving control of a saline solution of 9%, the second group 150 mg of alloxan was used to treat with administered peritoneal. The 400 mg of AHA / kg (body weight) was used treat the third group of mice while Aloxan and AHA were to treat the Group IV animals. The management of AHA aquatic extraction improved these criteria. These results indicated that AHA improves oxidative damages, hyperlipidemia and hyperglycemiain alloxan-induced diabetes in mice [24].

Three different types of buckwheat Spp. like *Fagopyrum cymosum, Fagopyrum tataricum* and *Fagopyrum esculentum* were tested for their anti-oxidant and antimicrobial potentials of volatile oils (VOs) extracts from their flowers. The hydro-distillation was used to obtain VOs of fresh buck wheat flowers and to analyze the chemical composition of extracts gas chromatography-mass spectrometry (GC-MS) was used. A remarkable antioxidant capacity of IC_{50}= 353.15 mg /mL, 264.92 gm /mL and 210.63 gm /mL from the 1.1-dichenyl 2-pycryl hydrazil (DPPH) measured as 174.13 g/mL, 243.16 gm/mL and 216.11 mg/mL, respectively was also shown by the VOs extracts from *F. cymosum, F. esculentum* and *F.tataricum* flowers, when

β-carotene-linoleic bleaching method was applied. Thus, the finding showed that the buckwheat flowers Vos extracts can be effectively used as natural antioxidants and antimicrobial agents [25].

The *Prunella vulgaris* Linn (*P. vulgaris*) was investigated for its antioxidant activity of several water-soluble polysaccharides extracts by using the DEAE-Sepharose flow column for different rinsing water (PV-P1), 0.2 M NaCl (PV-P3) and NaCl 0.1 M (PV-P2). As compared to PV-P2 and PV-P3A the higher degree of branching and a higher molecular weight was shown by Structural analysis of PV-P1. The all three extracts PV-P1, PV-P2 and PV-P3 against RA 264.7 in the tested concentrations no cellular toxicity was observed. So, it was shown by that common *P. vulgaris* polysaccharides can be inspected as possible antioxidant source, medicine, immunoglobulins or functional foods [26].

The antioxidant activity various extracts of dried *Polygonum aviculare* L. was investigated by using various assays by FRAP assay, lipid peroxidation and analysis of DNA-induced cleavage sequences. The IC_{50} value was measured by the results for different extracts which was 50 µg /mL, 0.9 µg /mL and 15 µg /ml for the DPPH antioxidant or radical scavenging assay, H_2O_2 superoxide radical assay and for lipid-peroxidation assay, respectively. Moreover, these extracts also showed a protective effect in hydroxyl radical-induced DNA strand assays. The value of TPC and TFC observed were 677.4 +/- 52.7 µg /g and 122.7 +/- 14 µg /g for these extracts. So, the significant antioxidant effects were shown by these findings of *Polygonum aviculare* L. extract [27].

Various species of Salvia medicinal plant e.g., *Salvia macrosiphon*, *Salvia sahendica, Salvia chloroleuca, Salvia xanthocheila, Salvia hydrangea, Salvia atropatana, Salvia ceratophylla, Salvia sclarea* and *Salvia glutinosa* species were evaluated for their antiproliferative and antioxidant potentials. The phytochemical properties, TPC and TFC were also observed. The highest antioxidant activity of IC_{50}= 8.20 mg^{-1} by *S. ceratophylla* was shown against C32 cells followed by *S. glutinosa* with an IC50 value of 5.29 mg^{-1} compared with ACHN cell lines. However, the *S. glutinosa* also showed higher uptake activity for DPPH roots with IC 50 than 3.2 μg^{-1}. The highest concentration of phenol and total flavonoids were shown by these species. So, these results specified the importance of salvia species as healthy plant foods [28].

The organo and thyme extracts were evaluated for their antioxidants effects in soybean oil is susceptible using thermal oxidation. About 3000 mg/kg of organo, thyme olease oil and their mixtures was found in Soybean oil, it also contains tributyl hydroquinone (TBHQ; 50 mg / kg) and soybean without oil exposed to thermal oxidation. Thus, physical, chemical and fatty acids were evaluated. A greater protective effect was applied by organo and thyme separately, which prevented the increase in the formation of TBHQ, showing that by adding of 3000 mg / kg ensures better protection against oxidative oxidation. The increased absorption of urea by adding the thyme and oregano extracts gave a greater protective effect [29].

The antioxidant potentials of various new plant like (*V. rhodopaea* L., *Veronica bellidioides* L., V. bccabunga L., *V. kellereri*, *V. Vindobonensis*, *V. austriaco*, *Clinopodium vulgare* L., *Stachysrecta* L., *Xeranthemum annuum* L. and *Clematis vitalba* were investigated in this research. The potential of antioxidants for new varieties comparable to plants reference drugs. This study showed the antioxidant potential and importance of various traditionally used medicinal plant species [30].

The antioxidant potentials of *C. tora* L. aqueous extracts were investigated in this study. It was noted that at a dose of 0.2 mg / mL, the *C. tora* (unroasted) showed 94% hang-up of linoleic acid peroxidation even greater than the alpha-tocopherol (82%). The roasting of water extracts of *C. tora* L. was achieved at 200 ° C for 5 minutes and 175 ° C for 5 minutes with inhibition of linoleic acid peroxidation results of 82% and 83%, respectively. The WEUCT-IC_{50} value was higher in the lipid formulations caused due to fenton reaction as 0.41 mg /mL, as compared to alpha tocopherol (IC_{50}) value of 0.55 mg/mL). Moreover, in the non-enzymatic and enzymatic systems of oxidization system, WEUCT also demonstrated a good anti-oxidant activity. The roasted *C. tora* L. aqueous extracts showed the high degree of staining resulting from coloring compared to the non-roasted sample [31].

The two local medicinal plant species *Saturja montana* L. and *S. subspicata* L. were investigated for eight potential phenolic components (p-coumaric, quercetin, rutin, protocatehúic, caffeine, rosmarinic, ellagic and jeringic acid). The HPLC of ethanolic and methanolic extracts was also measured. The chelating and radical-scavenging assays were used, and the results indicated that the polyphenols and other antioxidant compounds were possessed by both species. A wide range of antimicrobial activity was also observed for various microbial species tested *in-vitro* like (*Candida albicans*, *C. krusei*, *Microsporum gypseum*, *C. dubliniensis*, *C. glabrata*, *Staphylococcus aureus*, *C. parapsilosis* and *Escherichia coli*) by the extracts from both species [32].

The antimicrobial and antioxidant potentials of *Satureja montana* L. against seven species of bacteria were assessed here. It was observed that against *Salmonella typhimurium*, the ethanolic extracts were not effective and also no antibacterial activity was shown by water extracts. The main volatile components of essential oils were the thymol (141 g/L), carvacrol (306 g/L) and methyl ether (63 g /L) of these tested extracts. The hot water extracts of *S. montana* showed the strongest antioxidant capacity and also the essential oil with highest percentage of phenol of plant was measured. So, by these findings it was observed that the *S. montana* can be used as an efficient biologically active extract as natural antioxidants and antimicrobial source [33].

The antioxidant potentials of various extracts of *Galium odoratum* L. were evaluated in this study. The DPPH free radical scavenging assay was used to measure antioxidant potential of these extracts. The water extract were relatively better in Wound reduction and tissue standards (90.68 ± 6.13%, 97.18 ± 4.37% for water extracts 15% and 30% compared to 79.29 ± 9.16% and

91.94 ± 4.14% for 15% and 30% methanol extracts Respectively). The substantial antioxidant potential was noticed with IC_{50}=148 µg/mL and 83 µg/mL, for both methanol and aqueous extracts respectively, in the DPPH test. So, assumption was that both extractors have the potential antioxidant potential, as well as empirically and surgically demonstrated the relatively well-burned wound healing activity in the water extract [34].

The *Thymus serpyllum* L. was evaluated in this research for its total phenol content, antioxidant capacity of flavonoids, free radicals and potential effects of high blood pressure were studied for the water extract. The total phenol contents in TPC were measured as 2008.34 ± 10.5mg / L from GAE, and the rosmarinic and caffeic acids were the major phenolic compounds. The absorption activity of nitrogen oxide in vitro was 1 mg / l TE 63.43% showing an IC_{50} value of 124.40 µg/mL. It was noticed that in all experimental mice, after treatment with TE the heart index was same. No significant activity was shown by the dose given by TE in the uptake of nitric oxide in vivo. It was suggested by these results that TE can be used as antioxidant and also to protect from hyper-tension [35].

Almost 70 medicinal plant extracts were experimented for evaluation of their antioxidant potential and total phenol content (TPC). For human consumption infusions were prepared like tea. Folin-Ciocalteau test was applied to measure the TPC (total phenol contents) of the extracts. FRAP (Ferric Reducing Antioxidant Power) assay was followed to testify the total antioxidant potentials of these extracts. This medicinal plants infusion showed the total phenolic contents values in ranges from 10 to 2016 mg/L. The antioxidant activity was in range of 0.18 to 26 mm /L FRAP assay. The Phenolic of *M. folium* were highly effective ABTS free radical scavengers when compared to vitamin C and Trolox. Finally, from these findings the importance of *M. folium* can be concluded as a vital source of phenolic and antioxidant as compared to red wine or beverages such as tea [36].

Conclusion

In this experiment the free radical scavenging activity of various medicinal plant samples was measured using different extracts depending upon the ability to eliminate the free radicals using synthetic DPPH. The reactivity of different compounds with the stable free radicals was because of the odd number of electrons present in them. The results showed that ethanolic extracts of different medicinal plant samples have the higher level of free radical scavenging or antioxidant activity, followed by methanolic extracts comparing to the IC_{50}=37.337µg/mL value of the ascorbic acid(standard). The highest absorbance was observed in the *Euphrasiae stricta* (IC_{50}= 38.972 µg/mL), *Euphorbia platyphyllos* L. (IC_{50}= 40.817 µg/mL) and *Epimedium brevicomum* Maxim (IC_{50}= 46.265µg/mL), medicinal plants for both of their ethanolic and methanolic extracts. The total antioxidant potential of these medicinal plants was because of high amount of polyphenol and other phytochemical components found in them. The aqueous extracts showed almost similar and comparable results for all samples regarding free radical scavenging activity comparing with the Ascorbic acid. These finding also indicated that all tested medicinal

plants samples likely to possess significant level of free radical scavenging activity although comparatively less than standard ascorbic acid. So, this research suggested that all medicinal plants and particularly *Euphrasiae stricta*, *Euphorbia platyphyllos L* and *Epimedium brevicomum* Maxim possess a significant antioxidant potential and can be efficiently applied as an important antioxidant source for the treatment and inhibition of widely spreading oxidative stress related degenerative diseases like cancer, cardiovascular & inflammatory joint disorders, atherosclerosis, dementia, diabetes, asthma and eyes related degenerative diseases etc.

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